Colposcopy referral and CIN3+ risk of human papillomavirus genotyping strategies in cervical cancer screening

Running title: HPV genotyping strategies for colposcopy referral

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Conflict of interest: DAMH and CJLMM are minority shareholders of Self-screen B.V., a spin-off company of VUmc; Self-screen B.V. develops, manufactures and licenses high-risk HPV and methylation marker assays for cervical cancer screening and holds patents on these tests. DAMH has been on the speaker's bureau of Qiagen and serves occasionally on the scientific advisory boards of Pfizer and Bristol-Myers Squibb. CJLMM is part-time CEO of Self-screen B.V., and has a very small number of shares of MDXHealth and previously of QIAGEN, has received speakers fees from GSK, QIAGEN and SPMSD/Merck, and served occasionally on the scientific advisory boards (expert meeting) of these companies. KK, JAB, and JB declare no conflicts of interests.

Abbreviations: ASC-US: atypical squamous cells of undetermined significance; BMD: borderline or mild dyskaryosis; CI: confidence interval; CIN (2/3+): cervical intraepithelial neoplasia (grade 2/3 or worse); CISOE-A: composition, inflammation, squamous, other and endometrium, and endocervical cylindrical epithelium, and adequacy; DNA: deoxyribonucleic acid; (hr) HPV: (high-risk) human papillomavirus; HSIL: high-grade squamous intraepithelial lesions; LSIL: low-grade squamous intraepithelial lesions; (m)PPV: (marginal) positive predictive value; NILM: negative for intraepithelial lesion or malignancy; NPV: negative predictive value; PALGA: nationwide network and registry of histo- and cytopathology in the Netherlands; POBASCAM: Population-based Screening Study Amsterdam.

ABSTRACT

Background: High-risk human papillomavirus (hrHPV)-based cervical cancer screening program in the Netherlands led to a substantial increase in the number of colposcopy referrals and low-grade lesions. Genotyping strategies may be employed to lower the screening-related burden.

Methods: We evaluated fourteen triage strategies with genotyping (HPV16/18 or HPV16/18/31/33/45/52/58) for hrHPV-positive borderline or mild dyskaryosis (BMD) or normal cytology, using data from a population-based hrHPV-based screening trial with 5-year interval (POBASCAM). We considered colposcopy referral at baseline, after 6-month repeat cytology and after 5-year hrHPV testing. Performance was evaluated by one-round positive and negative predictive value (PPV and NPV) and two-round colposcopy referral rate. To identify efficient strategies, they were ordered by the one-round colposcopy referral rate. Adjacent strategies were compared by the marginal PPV for detecting one additional CIN3+ (mPPV).

Results: The most conservative strategy (repeat cytology after BMD and HPV16/18/31/33/45/52/58-positive normal cytology, next round otherwise) yielded an mPPV of 28%, NPV of 98.2%, and colposcopy rate of 47.2%. Adding direct referral after BMD or genotype-positive BMD yielded an mPPV≤8.2%, NPV≥98.5% and an increase in colposcopy rate of 1.9-6.5%. Adding direct referral after HPV16/18-positive normal cytology yielded an mPPV≤3.5%, NPV≥99.5% and an increase in colposcopy rate of 13.9%.

Conclusions: Direct colposcopy referral of women with BMD or normal cytology is unlikely to be efficient, but genotype-guided direct referral after BMD may be considered because the increase in colposcopies is limited.

Impact: HrHPV screening programs can become very efficient when immediate colposcopy referral is limited to women at highest CIN3+ risk.

Introduction

Human papillomavirus (HPV) is known to be the primary cause of cervical cancer[1]. Several countries, including the Netherlands, have transitioned from primary cytology to primary high-risk (hr) HPV-testing in their national cervical cancer screening programs[2]. This has led to an increase in the number of screen-positives, colposcopy referrals and detection of low-grade lesions. For example, when primary HPV-screening was implemented in the Netherlands in 2017, the number of screen-positives increased by 80% and the number of colposcopy referrals and low-grade lesions more than doubled[3]. Unnecessary referrals increase the costs of the screening program[4], overburden the healthcare system, create avoidable stress and anxiety in screened women[5], and increase the risk of obstetric complications[6].

With the goal of reducing unnecessary referrals in mind, the Netherlands has recently made two changes to their national hrHPV-based cervical cancer screening program[7]. Initially, between January 2017 and June 2022, all hrHPV-positive women with abnormal cytology were directly referred for colposcopy and women with normal cytology were invited for repeat cytology after 6 months. As of July 2022, women with borderline or mild cytological abnormalities (BMD) are directly referred only if positive for genotypes HPV16 or 18[8], and invited for repeat testing otherwise. Furthermore, the interval until the repeat test has been extended from 6 to 12 months. It is expected that this more targeted referral policy will reduce the number of referrals[9] while maintaining the effectiveness of the program to detect cervical intraepithelial neoplasia grade 3 or worse (CIN3+).

Extended genotyping to detect hrHPV types beyond 16 and 18 has not yet been implemented in the Dutch national cervical cancer screening program. In particular, the other five high-risk genotypes covered by the nonavalent HPV vaccine (31, 33, 45, 52 and 58[10]) are interesting to consider since three available extended genotyping tests (including BD Onclarity, which is used as the HPV test in the Netherlands from 2023, plus Alinity, and HPVIR[11–13]) allow for this combination as well as most full genotyping tests. Including extended genotyping for the management of hrHPV-positive women with normal or BMD cytology has the potential to further improve the effectiveness and/or efficiency of screening programs but could also add

complexity to the screening programs, so it must be determined whether the potential reduction in referrals is worth the added complexity.

In this study, we evaluated the performance of genotyping by types 16 and 18 (*HPV16/18 genotyping*) and genotyping by types 16, 18, 31, 33, 45, 52, and 58 (*extended genotyping*), in the management of hrHPV-positive women with baseline BMD or normal cytology. Specifically, we compared positive and negative predictive values (PPV and NPV) of different strategies by utilising an incremental benefit-harms framework originally developed for health technology assessments. For this purpose, we used data from the Population-based Screening Study Amsterdam (POBASCAM) - a population-based hrHPV screening study with multiple rounds of hrHPV-based screening, and full genotype results of hrHPV-positive samples. A unique feature of the POBASCAM study is that hrHPV-positive BMD and normal cytology are managed conservatively by repeat testing after 6 months. This enables us to compare colposcopy referral rates and CIN3+ rates of a wide variety of strategies with or without genotyping, including the strategies that were used in the Netherlands until and after June 2022.

Materials and methods

Study population

POBASCAM is a population-based randomized controlled trial (trial registration ID: NTR218) carried out in the setting of the national cervical cancer screening program in the Netherlands with enrolment between January 1999 and September 2002. The study design has been published before[14]. Briefly, 44,102 women aged 26-61 years attending routine cervical cancer screening were randomised (1:1) and managed by either cytology and hrHPV co-testing (intervention group) or conventional cytology (control group). In the intervention group, women with moderate dyskaryosis or worse (>BMD) cytology were referred for colposcopy, women with BMD cytology or an hrHPV-positive normal cytology result were referred to repeat testing, and women with an hrHPV-negative normal cytology result were redirected to routine screening. In the control group, women were managed according to the national screening protocol at that time, with colposcopy referrals based only on their cytology results while HPV test results remained blinded. Women with >BMD were referred for colposcopy, women with BMD were referred for repeat cytology and women with normal cytology were

redirected to routine screening. In the second screening round after 5 years, both groups were managed by cytology and hrHPV co-testing.

For our study, women who were hrHPV-positive and younger than 59 years old at baseline were included from both the intervention and control group, but women with >BMD cytology at baseline were excluded because their risk of CIN3+ warrants immediate referral in all strategies[15] (Figure 1). Furthermore, women with uterus extirpation or CIN2 in the first round were censored at the time of detection. Criteria for selecting women from the intervention or control arm were different for each triage strategy considered in our analysis, this is described in detail in the supplementary material.

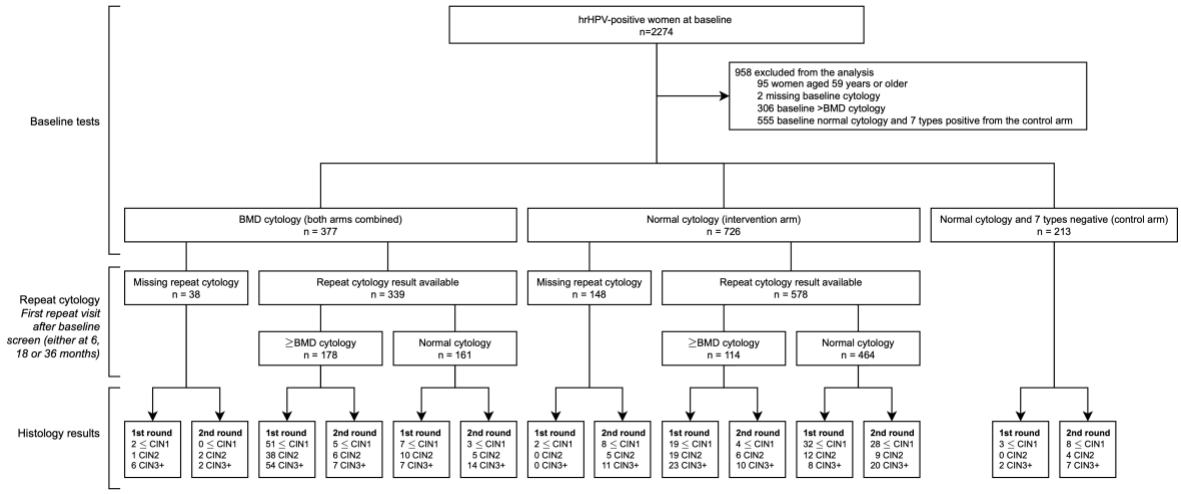

Figure 1. Flowchart of the POBASCAM women included in this analysis. "7 types" refers to the seven high-risk HPV types from the nonavalent HPV vaccine (HPV16/18/31/33/45/52/58).

Clinical information

Cervical smears were taken by a general practitioner or their assistant using a Cervex-Brush or cytobrush and brushes were stored in a vial containing 5ml phosphate-buffered saline collection medium for hrHPV testing. The HPV DNA tests (GP5+/6+ PCR-EIA) [16], which detected fourteen hrHPV genotypes (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), were blinded when reading cytology. Positive samples were subsequently typed using a reverse line blot assay[17]. The cervical smears were classified by cytotechnologists according to the CISOE-A framework used in the Netherlands[18]. Smears were classified as normal (Pap 1), BMD (Pap 2/3a1), and moderate dyskaryosis or worse (Pap 3a2/3b/4/5). These classifications can easily be converted into the Bethesda system (NILM, ASC-US/LSIL, and HSIL respectively), which is often used internationally to classify cytological smears[19]. After referral

to the gynaecologist, biopsies were taken at colposcopy visits from suspected areas according to standard procedures in the Netherlands[20,21]. Histology was examined locally and samples were classified as normal, cervical intraepithelial neoplasia (CIN) grade 1, 2, 3, or invasive cancer according to international criteria[22]. Adenocarcinoma in situ was added to CIN grade 3.

Screening strategies

In this study, fourteen triage strategies were compared. For the management of hrHPV-positive women with BMD cytology at baseline four strategies were considered, in order of decreasing aggressiveness: (1) all referred, (2) extended genotyping (for the 7 HPV types 16, 18, 31, 33, 45, 52, or 58) with immediate referral after a positive result for any of these seven HPV types and repeat cytology otherwise, (3) HPV16/18 genotyping with immediate referral after a positive result for either HPV-16 or -18 and repeat cytology otherwise, and (4) repeat cytology for all women. For the management of hrHPV-positive women with normal cytology at baseline five strategies were considered, in order of decreasing aggressiveness: (1) all referred, (2) extended genotyping with immediate referral after a positive result for any of the seven types mentioned above and repeat cytology otherwise, (3) HPV16/18 genotyping with immediate referral after a positive result for either HPV-16 or -18 and repeat cytology otherwise, (4) repeat cytology for all women, and (5) extended genotyping with repeat cytology after a positive result for any of the seven types mentioned above and routine screening otherwise. Combinations that involve more intensive management for normal cytology than for women with BMD cytology were excluded.

The combination of strategy 1) for BMD and 4) for normal cytology was used in the Netherlands until mid-2022 and is our reference strategy (labelled *R*). Seven strategies are more conservative than R (labelled *C1-7*) and six are more aggressive (labelled *A1-6*). Of note, the combination of strategy 3) for BMD and 4) for normal cytology (labelled C4) is very similar to the current triage strategy in the Netherlands except that repeat testing in our analysis is done after 6 instead of 12 months.

For the second-round referrals, we added all women who had not been referred yet but who were still hrHPV-positive in the second round of POBASCAM, since it has been shown that women with five-year HPV persistence have a high CIN3+ risk[23]. At baseline and at the repeat

test after 6 months, all our proposed strategies are at least as aggressive as the POBASCAM strategy. However, the POBASCAM study has a second repeat hrHPV test at 18 months which enables us to estimate the percentage of missed CIN3+ in the first round by each of the strategies.

Statistical methods

The performance of fourteen triage strategies was evaluated by i) cumulative colposcopy referral rate at baseline, after the repeat test and after two rounds of screening, ii) PPV for detection of CIN3+ in the first round, and iii) NPV for CIN3+ in the first and second round.

Colposcopy referrals were adjusted for loss to follow-up by means of a Bayesian framework with Jeffrey's prior[24] for the binomial referral rate distribution. Confidence intervals for the referral rate were calculated with the equally-tailed Jeffrey's interval. Loss to follow-up and absence of genotyping results was accounted for by imposing a missing at random assumption for women with incomplete follow-up where the probabilities of positive cytology and HPV results at 6 months and at 5 years were stratified by previous cytology and HPV results. In the POBASCAM study, 8.4% of women with baseline BMD cytology and 20.2% of women with normal cytology (from the intervention group) had missing repeat test results. Additionally, of the women who were not referred in the first round of POBASCAM according to the study protocol, 19.7% of women with BMD cytology at baseline and 15.6% of women with normal cytology had missing 5-year follow-up test results.

PPV and NPV for the detection of CIN3+ were calculated from cross-tables of referrals and histology outcomes. For these calculations, we did not adjust for loss to follow-up, instead we used crude numbers of CIN3+ cases that would have been detected by each strategy. We used both arms of POBASCAM for women with BMD cytology and only the intervention arm for women with normal cytology. PPV estimates were stratified by baseline cytology and presented with 95% Wilson score confidence intervals. For the calculation of NPV, results were not stratified by baseline cytology, hence the normal cytology cases in the data were doubled so that the correct distribution of baseline cytology is maintained in the population. Confidence intervals for the NPV were calculated using bootstrap methods. The PPV and NPV are common measures of the potential harms and safety of screening[25]. In the Netherlands, an informal criterion of an acceptable strategy is that the NPV for the detection of CIN3+ is at

least 98% and the PPV is at least 20%[26]. Lastly, to identify strategies that provide an optimal balance of the number of colposcopy referrals and the number of CIN3+ detected in the first round, we performed an incremental analysis. We utilised the incremental cost-effectiveness framework originally developed for health technology assessments. We set costs equal to the number of colposcopy referrals and effects equal to the number of CIN3+ detected and ordered strategies according to number of colposcopy referrals. The cost-effectiveness of a strategy compared to an adjacent non-dominated strategy with lower costs (i.e. fewer colposcopy referrals) is measured by the incremental cost-effectiveness ratio (ICER). Notably, a strategy is called non-dominated when there does not exist another strategy or combination of two other strategies that yields a higher number of CIN3+ against the same number of referrals. We used the inverse of the ICER which is the extra number of CIN3+ detected per extra colposcopy referral (marginal PPV, mPPV). The curve connecting non-dominated strategies in a two-dimensional plot of number of colposcopy referrals against number of CIN3+ is the efficient frontier[27]. To select strategies on the efficient frontier, an mPPV threshold of 20% was used by analogy with the informal Dutch PPV threshold. All statistical analyses were performed using R software (version 4.2.3)[28]. The estimation of the colposcopy referral rates is described in more detail in the supplementary material.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Characteristics of study population

Table 1 presents the distribution of age, baseline cytology, hrHPV genotype and histology results stratified by baseline cytology in the subsets of the POBASCAM intervention and control group used in our analysis. Three-hundred seventy-seven hrHPV-positive women with baseline BMD cytology from both arms of the POBASCAM study were included in our analysis. Mean age of these 377 women was 35.9 (range 29 – 55) years. Of these women, 160 (42.4%) were positive for hrHPV genotypes 16 or 18, 123 (32.6%) were negative for HPV16 and 18 but positive for one or more of the high-risk types 31, 33, 45, 52, and 58, and 94 (25%) were only positive for a different hrHPV genotype. Sixty-seven (17.8%) of the 377 women

had CIN3+ in the first round of the study, and 109 (28.9%) had histology results that were <CIN3 in the first round. One-hundred fifty-seven (41.6%) did not have any histology results reported. In the second round of POBASCAM, twenty-three (6.1%) of the 377 women had CIN3+ and 21 (5.6%) had histology results that were <CIN3.

Seven-hundred twenty-six hrHPV-positive women with normal cytology from the intervention group were included in our analysis. Mean age of these women was 37.4 (range 29 – 58) years, 252 (34.7%) were positive for HPV 16 and/or 18, 253 (34.9%) were negative for HPV16 and 18 and positive for one or more of the five extended high-risk types and 221 (30.4%) were only positive for a different hrHPV genotype. Of these women, 31 (4.3%) and 84 (11.6%) had CIN3+ and <CIN3 reported in the first round, respectively. Forty-one (5.7%) had CIN3+ in the second round, 60 (8.2%) had histology results that were <CIN3 in the second round, and 510 (70.2%) never had any histology results reported.

Lastly, 213 hrHPV-positive women who were negative for the 7 types (16/18/31/33/45/52/58) with normal cytology from the control group of POBASCAM were included in our analysis for strategies that immediately dismiss women until the next round. Mean age of these women was 38.8 (range 29 – 55) years. Of these women, 2 (0.9%) and 3 (1.4%) had CIN3+ and <CIN3 reported in the first round, respectively. Seven (3.3%) had CIN3+ in the second round, 12 (5.6%) had <CIN3 in the second round, and 189 (88.7%) had no histology results ever reported.

Colposcopy referrals in the first and second screening round

The colposcopy referral rates of the fourteen triage strategies are shown in Figure 2 and Table S1. The reference strategy (also highlighted in grey in Table S1) had a first-round colposcopy referral rate of 36.0% (95% CI: 32.4 - 39.8) and cumulative two-round colposcopy referral rate of 54.1% (48.6 - 59.9). Strategies that are more aggressive than the reference strategy had a higher first round referral rate as illustrated in Figure 2 ranging from 50.7% (95% CI: 46.5 - 55.0) to 100% (95% CI: 96.3 – 100.0). The higher referral rate persisted over two rounds when all persistent hrHPV infections in the second round were also referred (Figure 2). The strategies that were more conservative than the reference strategy showed substantially lower referral rates in the first round (Figure 2). However, the decrease in the colposcopy referral rate became smaller after the repeat and the second round hrHPV test. Compared to

the most conservative strategy (C7: repeat cytology after BMD and HPV16/18/31/33/45/52/58-positive normal cytology, 5-year hrHPV testing otherwise), adding direct referral after HPV16/18-positive BMD increased the immediate colposcopy referral rate by 8.3%, but the two-round colposcopy rate only by 1.9%, and adding direct referral after all BMD increased the immediate colposcopy referral rate by 20.5%, but the two-round colposcopy rate only by 6.5%. Further adding direct referral after HPV16/18-positive normal cytology increased the two-round colposcopy referral rate by another 13.9% (95% CI: 5.9 – 21.9).

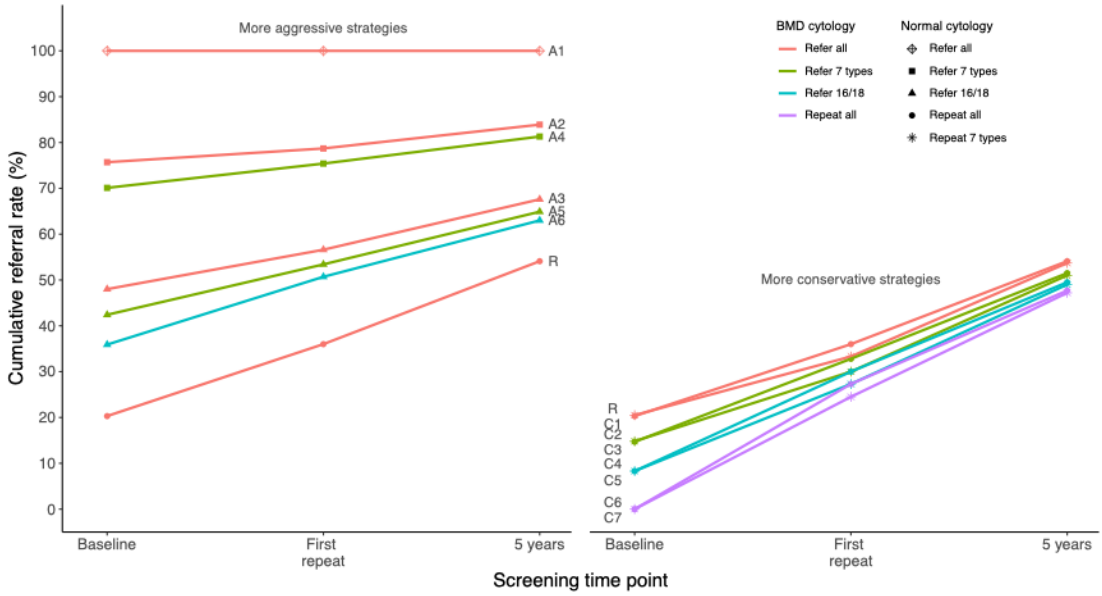

Figure 2. Cumulative referral rate for fourteen genotyping strategies over three screening time points (baseline, first repeat test and second round)

PPV and NPV in the first and second screening round

Detailed results of the performance of the fourteen strategies in terms of PPV and NPV are given in Table 2 and 3, respectively. For BMD baseline cytology, the 20% PPV threshold was met at baseline when HPV16/18 or 7 types positive were referred, but for the latter strategy the PPV at the repeat test was lower than 20% (15.6%, 95% CI 6.9 - 31.8). For normal baseline cytology, the PPV at baseline and at the repeat test fell below 20% for strategies where women were immediately referred. The PPV of HPV16/18 triage after normal cytology was 10.3% (95%CI: 7.1 – 14.7) for referrals at baseline and 6.6% (95%CI: 2.6 – 15.7) for referrals after repeat cytology. Only for the four most conservative strategies (C4, C5, C6, and C7) the PPV at baseline and after the 6-month repeat test was at least 20% for baseline BMD and normal cytology strata.

For the second round hrHPV test, PPV of the most aggressive strategy (A1) was 26.9% (95% CI, 13.7 - 46.1) in the subgroup with baseline BMD cytology and 25.4% (95% CI, 16.7 - 36.6) for the subgroup with normal baseline cytology. This PPV lay above 20% and was even higher for the other, more conservative strategies.

In the first round, all strategies had an NPV above 98%, ranging from 98.2% (C7; 95% CI, 97.5 - 98.9) to 100% (A2; 95% CI, 99.4 – 100.0 and A4; 95%CI, 98.8-100.0). Strategies with direct referral after HPV16/18-normal cytology had an NPV of at least 99.5% (A6; 95%CI 99.0-99.9). For two rounds cumulatively, the NPVs were lower, ranging from 93.7% (C7; 95% CI, 91.3 - 94.0) for the most conservative strategy to 98.1% (A4; 95% CI, 96.8 - 99.3) for the strategy where women positive for the 7 types were immediately referred.

Incremental analysis

To further discriminate between the different strategies on the basis of the performance in the first screening round, an efficient frontier is shown in Figure 3. Seven of the fourteen strategies lay on the efficient frontier. None of the strategies that used the same strategy for normal and BMD cytology lay on the efficient frontier, supporting the use of different criteria for immediate referral in BMD and normal cytology results. The only strategy with an mPPV above 20% was C7 with an mPPV of 28% (95% CI, 23.7 - 32.8). The next strategy, C5, had an mPPV of 8.2% (95%CI, 3.8 - 16.8). Other strategies had an mPPV below or equal to 5%, including A6 with direct referral after HPV16/18 normal cytology (mPPV 3.5%, 95% CI 2.1 – 5.8).

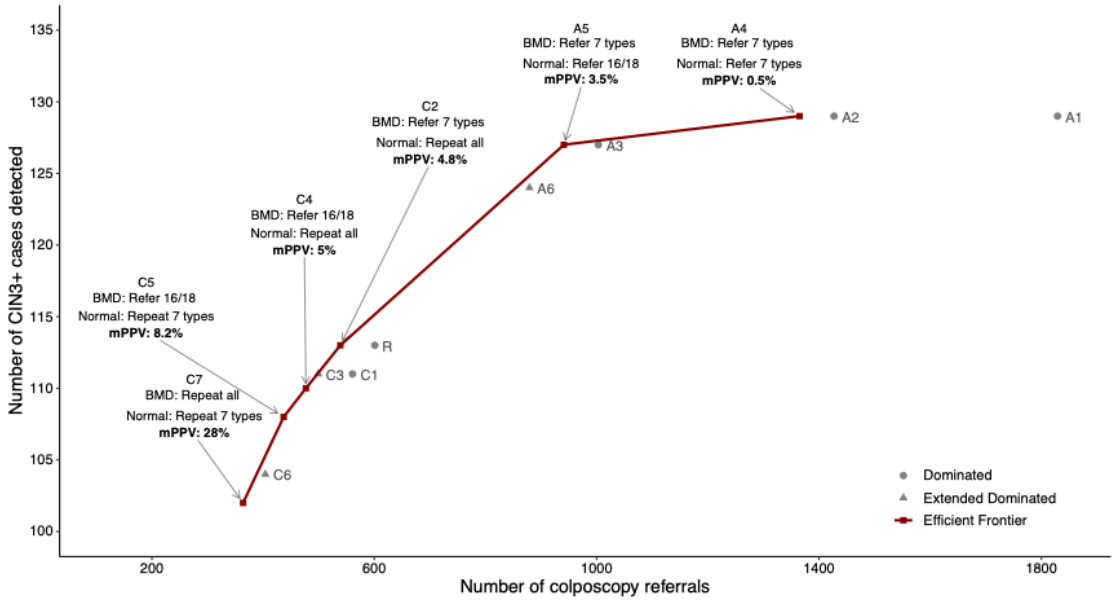

Figure 3. Efficiency plot of the number of first round referrals against the number of **first round** CIN3+ cases detected for each of the fourteen strategies. The efficient frontier is shown in red, with labels specifying the efficient strategies and their respective marginal PPV (mPPV).

Discussion

In this analysis we compared fourteen strategies with cytology and/or HPV genotyping for the management of hrHPV-positive women in cervical cancer screening programs. We did not include women with severe cytological abnormalities, as immediate colposcopy is indicated for this group because of a very high CIN3+ risk. We considered the primary HPV-based strategy that was used in the Netherlands between 2017 and 2022, referred to as the reference strategy, plus six more aggressive and seven more conservative strategies than the reference strategy.

In our incremental analysis we showed that starting from a strategy where all women with BMD or HPV16/18/31/33/45/52-positive normal cytology were advised repeat testing (C7), strategies with direct referral for subgroups of women led to only a small increase in detected CIN3+, reflected by a low mPPV of 8.2% to detect one CIN3+ among additionally referred women and only a minor increase of 0.3-0.5% in NPV for CIN3+ detected in the first round. This value was lower than our benchmark mPPV value of 20%[26] and suggests that directly referring a subgroup of women based on HPV genotyping is not efficient.

Nevertheless, direct referral for subgroups of women with BMD at baseline may be considered because it did not lead to a strong increase in colposcopy referral rates, at least when evaluated after repeat cytology testing or second round hrHPV testing. Another argument for direct referral of BMD subgroups is a concern about interval cancers, in particular when the sample is positive for HPV16/18 since these types are associated with the majority of cervical cancers. In the POBASCAM trial, there were five cancers detected in the first round after BMD cytology[29]. All of them were detected by repeat cytology at 6 months and four of them had HPV16/18 at baseline. A third argument for direct referral of BMD subgroups is that repeat testing may lead to loss to follow-up and induces extra screening costs although these costs are expected to be substantially lower than costs incurred after colposcopy referral.

Our incremental analysis did not give favourable results for immediate referral of subgroups of women with normal cytology at baseline since the mPPVs lay below 4%. Besides, directly referring a subgroup of women with normal cytology resulted in a considerable increase in referral rate compared to the reference strategy, also cumulatively over two screening rounds. Such a policy is not acceptable for the Netherlands, also because it leads to the detection of many low-grade lesions[3], but it has been proposed in many other settings. In the literature, a widely proposed strategy with genotyping is to refer women with HPV16/18 immediately and to refer women with another HPV genotype when cytology is abnormal[33–36] which is equivalent to our strategy A3. Similar as for HPV16/18-positive BMD, an important argument for direct referral based on HPV16/18 genotyping in those studies is that these two genotypes are associated with the highest risk of cervical cancer and have been detected in around 70% of cervical cancers[37], justifying immediate referral of HPV16/18-positive women without cytology. Such a strategy might be particularly interesting when the quality of cytology is low, although in those settings one may also consider alternatives to cytology such as for instance host cell DNA methylation[38] in combination with HPV genotyping, and proposal triage strategies are emerging[39,40].

In our study, we used an NPV threshold for detection of CIN3+ of 98%, or equivalently, a CIN3+ risk threshold of 2%. This informal consensus threshold[26] is loosely based on a CIN3+ risk of 1.2% in women with BMD cytology at baseline and two normal repeat tests in the previous cytology-based program[31]. We used a PPV consensus threshold of 20%[26], which is based on the previous cytology-based program where the PPV of abnormal cytology was 20-30%[31]. However, it is important to underline that NPV and PPV thresholds are country-specific and depend on country-specific health resources and priorities, similar as decisions on the screening interval and start and end age. The Netherlands has a relatively low number of screening rounds which leads to a relatively high PPV and low NPV and thresholds need to take this into account. Other countries often use a lower threshold for the PPV and a higher threshold for the NPV. The US, for example, recommends an NPV and PPV threshold of 99.85% and 4% respectively[32].

The POBASCAM cohort only consisted of unvaccinated women because enrolment was before national HPV vaccination was introduced. In future vaccinated cohorts, HPV16/18 and HPV16/18/31/33/45/52/58 positivity are expected to decrease in BMD and normal cytology.

Then the impact of genotyping on colposcopy referral rate will be smaller (although the mPPV will likely remain the same) and genotyping may be considered also for women with normal cytology when it does not markedly influence the colposcopy referral rate.

This study has both strengths and limitations. The main strength of this analysis is the use of the POBASCAM study, which followed approximately 40,000 women aged 29 to 60 over two screening rounds (9 years). A unique opportunity provided by the POBASCAM study is that hrHPV-positive women with normal and BMD cytology are managed conservatively by repeat testing and full genotyping has been conducted on all hrHPV-positive samples. This enabled us to evaluate the performance of potential genotyping triage strategies for hrHPV-positive women. Additionally, the POBASCAM study was embedded in the national screening program, and the cytology and HPV genotype distribution in hrHPV-positive women are fairly similar in the POBASCAM study and in the primary HPV national screening program in 2017 despite the 15 years' time difference: the percentages of BMD and > BMD were 17% and 14% in the POBASCAM study[14] and 21% and 11% in the national program[41] and HPV16/18 percentages were 43% in the POBASCAM study[42] and 34% in the national program[41].

We identified the following limitations of our study. First, in the POBASCAM study, cytology was performed without knowledge of the hrHPV status. This is different from the current programme where the HPV status is known since only hrHPV-positive women have a smear taken, which might influence the results. A recent study by Kholova and colleagues[43] compared two rounds of cytopathological diagnoses where the HPV status was only known in the second round. By comparing the results of the two rounds, they showed that agreement was better when the HPV status was known. Second, not all women had repeat test results available. For the calculation of colposcopy referral rates, loss to follow-up was corrected for by using a missing at random assumption for women who did not attend repeat testing and stratifying the probabilities of positive cytology and HPV results at 6 months and at 5 years by previous cytology and HPV results. For the calculation of CIN3+ numbers and mPPV, we did not adjust for loss to follow-up but adopted an intention to detect and treat approach in order to reflect clinical practice as closely as possible. In the POBASCAM study, the compliance with follow-up testing was higher than 80% so that 10-30% higher PPVs are expected after adjusting for loss to follow-up[29]. Third, histological diagnoses were made by a local pathologists as they were retrieved from the nationwide network and registry of histo-

and cytopathology (PALGA). However, we earlier showed by reviewing slides by two experienced pathologists that local CIN3 diagnoses achieved high accuracy in the POBASCAM study[29].

Conclusion

Overall, adding partial or extended genotyping to primary hrHPV-based screening programs is a trade-off between yield (CIN3+ detection) and potential harms (colposcopy referrals). Immediate referral of women with normal cytology is not recommended, but immediate referral of women with BMD or genotype-specific subgroups of BMD may be considered because it only leads to a limited increase in number of colposcopies. However, the gain in CIN3+ detection is also limited asking for a judicious assessment of the added value of genotyping.

Additional Information

Authors' contributions: Kelsi R. Kroon: Conceptualization, Formal analysis, Methodology, Visualisation, Writing - Original Draft Preparation; **Johannes A Bogaards**: Conceptualization, Supervision, Writing - Reviewing and Editing; **Daniëlle AM Heideman**: Data curation, Writing-Reviewing and Editing; **Chris JLM Meijer**: Data curation, Writing - Reviewing and Editing; **Johannes Berkhof**: Conceptualization, Supervision, Writing - Reviewing and Editing. All authors gave final approval of the submitted version of the manuscript. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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Tables

Table 1. Population characteristics of the intervention and control arm of the POBASCAM study

	BMD cytology (%)	Normal cytology (intervention group) (%)	Normal cytology (7 types negative & control group) (%)
Total	377	726	213
Age			
29-33	153 (40.6)	246 (33.9)	58 (27.2)
34-38	110 (29.2)	209 (28.8)	53 (25.0)
39-43	45 (11.9)	95 (13.1)	35 (16.4)
44-48	35 (9.3)	67 (9.2)	28 (13.1)
49-53	24 (6.4)	57 (7.8)	22 (10.3)
54-58	10 (2.6)	52 (7.2)	17 (8.0)
HPV genotype			
16/18	160 (42.4)	252 (34.7)	-
31/33/45/52/58	123 (32.6)	253 (34.9)	-
Other	94 (25.0)	221 (30.4)	213 (100)
Histology			
(1st round) CIN3+	67 (17.8)	31 (4.3)	2 (0.9)
(1st round) <CIN3	109 (28.9)	84 (11.6)	3 (1.4)
(2nd round) CIN3+	23 (6.1)	41 (5.7)	7 (3.3)
(2nd round) <CIN3	21 (5.6)	60 (8.2)	12 (5.6)
No Histology	157 (41.6)	510 (70.2)	189 (88.7)

Table 2. PPV for CIN3+ of 14 different strategies in hrHPV-positive women stratified by baseline cytology

	Baseline BMD cytology	Baseline normal cytology	Baseline PPV (95% CI)		Repeat cytology PPV (95% CI)	
			BMD cytology	Normal cytology	BMD cytology	Normal cytology
A1	**All** referred	All referred	17.8 (14.2 - 22.0)	4.3 (3.0 - 6.0)	--	--
A2		Refer 7 types [a] positive	17.8 (14.2 - 22.0)	5.9 (4.2 - 8.3)	--	5.0 (0.9 - 23.6)
A3		Refer 16/18 positive	17.8 (14.2 - 22.0)	10.3 (7.1 - 14.7)	--	6.6 (2.6 - 15.7)
R		Repeat all types	17.8 (14.2 - 22.0)	--	--	20.5 (14.1 - 28.9)
C1		Repeat 7 types only	17.8 (14.2 - 22.0)	--	--	23.9 (16.4 - 33.6)
A4	**7 types**	Refer 7 types positive	21.9 (17.5 - 27.1)	5.9 (4.2 - 8.3)	15.6 (6.9 - 31.8)	5.0 (0.9 - 23.6)
A5	Pos: refer	Refer 16/18 positive	21.9 (17.5 - 27.1)	10.3 (7.1 - 14.7)	15.6 (6.9 - 31.8)	6.6 (2.6 - 15.7)
C2	Neg: repeat	Repeat all types	21.9 (17.5 - 27.1)	--	15.6 (6.9 - 31.8)	20.5 (14.1 - 28.9)
C3		Repeat 7 types only	21.9 (17.5 - 27.1)	--	15.6 (6.9 - 31.8)	23.9 (16.4 - 33.6)
A6	**16/18**	Refer 16/18 positive	28.1 (21.7 - 35.5)	10.3 (7.1 - 14.7)	20.4 (13.5 - 29.7)	6.6 (2.6 - 15.7)
C4	Pos: refer	Repeat all types	28.1 (21.7 - 35.5)	--	20.4 (13.5 - 29.7)	20.5 (14.1 - 28.9)
C5	Neg: repeat	Repeat 7 types only	28.1 (21.7 - 35.5)	--	20.4 (13.5 - 29.7)	23.9 (16.4 - 33.6)
C6	Repeat **all**	Repeat all types	--	--	32.6 (26.2 - 39.7)	20.5 (14.1 - 28.9)
C7		Repeat 7 types only	--	--	32.6 (26.2 - 39.7)	23.9 (16.4 - 33.6)

[a] *7 types* refers to the seven high-risk HPV types (16, 18, 31, 33, 45, 52, and 58) from the nonavalent vaccine

Table 3. NPV for CIN3+ of 14 different strategies in hrHPV-positive women

	Baseline BMD cytology	Baseline normal cytology	NPV (first round) (95% CI)	NPV (cumulative 2 rounds) (95% CI)
A1	**All** referred	All referred	--	--
A2		Refer 7 types [a] positive	100.0 (100.0 - 100.0)	98.0 (96.7 - 99.3)
A3		Refer 16/18 positive	99.8 (99.4 - 100.0)	96.4 (95.1 - 97.6)
R		Repeat all types	98.7 (98.1 - 99.3)	94.3 (93.0 - 95.6)
C1		Repeat 7 types only	98.6 (97.9 - 99.3)	94.2 (92.9 - 95.5)
A4	**7 types**	Refer 7 types positive	100.0 (100.0 - 100.0)	98.1 (96.8 - 99.3)
A5	Pos: refer	Refer 16/18 positive	99.8 (99.5 - 100.0)	96.5 (95.3 - 97.7)
C2	Neg: repeat	Repeat all types	98.8 (98.1 - 99.4)	94.5 (93.3 - 95.8)
C3		Repeat 7 types only	98.7 (98.0 - 99.3)	94.3 (93.1 - 95.6)
A6	**16/18**	Refer 16/18 positive	99.5 (99.0 - 99.9)	96.2 (95.0 - 97.5)
C4	Pos: refer	Repeat all types	98.6 (97.9 - 99.2)	94.4 (93.2 - 95.6)
C5	Neg: repeat	Repeat 7 types only	98.5 (97.9 - 99.1)	94.2 (93.0 - 95.5)
C6	Repeat **all**	Repeat all types	98.3 (97.6 - 99.0)	93.3 (92.0 - 94.6)
C7		Repeat 7 types only	98.2 (97.5 - 98.9)	93.2 (92.0 - 94.5)

[a] *7 types* refers to the seven high-risk HPV types (16, 18, 31, 33, 45, 52, and 58) from the nonavalent vaccine